PRESENTATION OF THE ACADEMY MEDAL TO LLOYD OLD, M.D.*

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IT is with great pleasure and pride that I approach my assignment this evening to talk about the work of Dr. Lloyd Old before presenting him the New York Academy of Medicine medal for 1984. We first met in 1958 when we both arrived at the Memorial Sloan-Kettering Cancer Center, attracted by the Center's reputation for excellence in cancer therapy and cancer research. It was not until the mid-1960s, however, that I began to work closely with him in his chosen field of research, tumor immunology.

During the last two decades there has been a great resurgence of interest in approaches to cancer based on immunology. Such approaches have been based on the belief that there is something unique about a cancer cell that distinguishes it from normal cells, and that this difference can be recognized by the body's immune system. Of all the parts of the living cell that might be altered when it becomes cancerous, the surface of the cell is most suspect. Many of the regulatory signals that control the growth and multiplication of cells have their primary site of action at the cell surface, and it is easy to visualize that subtle changes in the surface structure of cells may have profound effects on their behavior. A thread that connects many aspects of Lloyd Old's work is the contention that the cell surface holds many of the keys to cellular differentiation and morphogenesis, and to the aberrations of these processes which are characteristic of cancer.

A major topic that has occupied Lloyd Old for the past 25 years is the serological analysis of the surface antigens of cancer cells. Although cancer-specific antigens were what he set out to find, he discovered that the study of cancer cells reveals important and surprising things about normal cells. The antigens he discovered led him to inquire into aspects of gene regulation and differentiation, cellular and transplantation immunology and viral biochemistry and genetics. As he uncovered the rich diversity of antigens

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expressed on the surface of cancer cells, he revised some of the preconceived ideas about what characteristics cancer-specific antigens would or should have. His studies of mouse leukemia have been especially rewarding in this regard. In the mouse this cancer generally originates in the thymus gland, which provides the serologist with an ideal opportunity to compare the surface antigens of leukemia cells with those of normal thymocytes. In serological studies beginning in 1961, Lloyd Old and his colleagues defined a host of cell surface antigens of mouse leukemia cells and normal thymocytes so that more is now known about the surface antigens of these cells than about those of any other cell types.

On the basis of these serological studies, the cell surface antigens of mouse leukemia cells can be grouped into several major categories. The first is exemplified by a series of antigens, called Lyt antigens, that mark all lymphoid cells derived from the thymus. These antigens have been termed differentiation antigens by Old and Boyse, because the genes controlling their expression are activated only in cells following a particular pathway of differentiation. Thus, leukemia cells of thymic origin have these antigens as a direct result of their cellular ancestry. Although initially detected on mouse leukemia cells, these antigens have been of considerable interest to immunologists in general since the subsequent discovery by Lloyd Old and his col-

leagues that three classes of T lymphocytes with different immunological functions —helper cells, suppressor cells and cytotoxic cells—can be distinguished by the patterns of Lyt antigens expressed on their surface.

The second category of surface antigens on mouse leukemia cells are viral antigens that can be traced to the mouse leukemia virus. Viral leukemia antigens were found on the surface of leukemia cells, on normal thymocytes of mice bred for a high incidence of leukemia and on cells intentionally infected by mouse leukemia virus. These antigens are protein components of the mouse leukemia virus particle. When complexed with carbohydrate and inserted into the cell membrane, they become cell surface antigens. Some of these antigens appear only when virus is being actively produced, but others may also appear on the normal thymocytes of certain mouse strains in the absence of virus production. This observation suggested that mouse leukemia virus genes are incorporated into the genetic material of all mice but are expressed to varying degrees. Thymic differentiation is one factor supporting expression in certain strains of mice, placing these viral gene products into the class of differentiation antigens in this case. On the other hand, appearance of these viral antigens in leukemias of mice whose normal thymocytes do not express these antigens puts them into the category of tumor-specific antigens in other strains of mice.

The third category of mouse leukemia cell surface antigens defined by Lloyd Old and his colleagues has been referred to as derepression antigens, the TL system first described in 1963 being the classical example. The antigen is called TL because the only types of cells on which it is found are normal mouse thymocytes and leukemia cells. Normal mouse strains can be classified at TL-positive or TL-negative on the basis of typing the thymus for the antigen. The characteristic feature of TL is that when leukemia arises in mice that lack TL on their thymocytes, the leukemia cells may express it. This has been explained on the grounds that leukemia brings about a change in the genes that repress TL expression, resulting in the anomalous appearance of TL on the surface of the leukemia cell. These findings have raised interesting questions about the antigens cancer immunologists classify as tumor-specific. Some of them can be considered tumor-specific under conditions where they appear in mice that in normal life never express them. Yet these antigens are not truly tumor-specific in the sense that they occur only on cancer cells, because they can also occur as components of normal cells in other strains of mice.

In contrast to mouse leukemia cells, the cell surface antigens of another

class of tumors induced in experimental animals—sarcomas induced by chemical carcinogens—have long defied serological analysis. This has been particularly frustrating because each of these tumors can be shown, in transplantation experiments, to elicit immunity to itself but not to any other tumor. Even when two tumors are induced by the same carcinogen in the same animal, each can be demonstrated to have distinct transplantation antigens, a finding that provides the firmest evidence in experimental animals for the existence of tumor-specific antigens. There has been much speculation regarding the genetic basis for the remarkable diversity of this class of tumor antigens, but the enigma remains essentially unsolved. Biochemical characterization of these antigens has been difficult primarily because the techniques to detect them, transplantation experiments, are cumbersome and slow. Lloyd Old and his colleagues, persevering in efforts to produce serological probes, have succeeded during recent years in developing antibodies that detect these individually specific antigens of chemically induced sarcomas, opening the way for structural and genetic studies which are now underway.

With the foundations of experimental tumor immunology laid during the 1960s, Lloyd Old turned to the question of human cancer antigens. These studies began with the search for antibodies in patients' sera reacting with antigens of established lines of human cancer, and the first target was Burkitt's lymphoma cells, which had just been shown to contain a virus, the Epstein-Barr virus. Lloyd Old discovered that patients with another much more common cancer, nasopharyngeal carcinoma, in addition to patients with Burkitt's lymphoma, had high titers of antibodies against the Epstein-Barr virus in their blood, thus linking the virus to a cancer that could not have been suspected of a viral etiology on any other grounds. Regarding serological reactions with the majority of cancers that did not contain any known virus, it turned out to be very difficult to analyze their specificity, because antibodies to blood group and histocompatibility alloantigens interfered with the detection and analysis of antibodies to other classes of tumor antigens that might be present. Autologous combinations of tumor cells and sera from the same patient would of course eliminate reactions to allogenic surface antigens, but this would mean that tumor cell lines would have to be established from each patient rather than having the convenience of using standard tissue culture cell lines that had already been established. Establishing cell lines of human cancers is not an easy task. Melanoma, astrocytoma and renal cancer are exceptions in that 20 to 30% of tumor specimens can be established and maintained as permanent tissue culture lines. It is for this

reason that Lloyd Old initially stressed these tumor types in his studies of autologous cancer immunity. What has emerged from these studies is a method to analyze autologous serological reactivity, a method referred to as autologous typing. Autologous typing provides the most direct and unambiguous way to ask questions about the humoral immune response to cancer in humans. The essential features of autologous typing are cultured tumor cells and serum specimens from the same patient, which are tested for reactivity with surface antigens of the autologous tumor target. The specificity of positive reactions is then analyzed by absorption tests on an extensive panel of autologous and allogeneic cells, both normal and malignant. In this way, with hundreds of individual cancers established in tissue culture, Lloyd Old defined several classes of surface antigens on human cancer cells. Some antigens are restricted to the autologous tumor cell; they are not found on any other normal or malignant cell type, and can therefore be considered the extreme of tumor-specific antigens—found on only a single tumor. Other antigens are shared tumor antigens that are found on autologous as well as allogeneic tumor cells; in some cases these antigens are found on a restricted set of normal cell types and can therefore be considered autoantigenic differentiation antigens; in other cases they have not as yet been found on any normal cell type. In contrast to the absolute or relative restriction of these categories of antigens to cancer, other antigens were found widely distributed on malignant as well as on normal cells. The largest number of reactions detected by autologous typing were in fact directed against the latter category of antigens, and such antibodies in addition to alloantibodies undoubtedly account for the great majority of positive reactions recorded in past serological studies of human cancer and for many of the mistaken claims for tumor-specificity. Now that clear lines have been drawn by the approach of autologous typing, one question which is being pursued is whether the production of antibodies against the interesting cancer-restricted antigens can be induced by immunization and whether the antibody response, if it can be induced, is associated with a favorable clinical course.

During the late 1970s the serological analysis of human cancer was revolutionized by a remarkable technical advance, the advent of the hybridoma methodology. Using mouse monoclonal antibodies produced by that technique, Lloyd Old and his colleagues have undertaken the most comprehensive analysis of human cancer cell surface antigens anywhere in recent years. Although it was the search for tumor-specific antigens that motivated much of the effort, tumor-specific antigens have not been found. Rather, a large

number of new differentiation markers has been recognized, some of them showing remarkable restriction to particular pathways of differentiation. The study of differentiation markers on human cancer is rapidly becoming an area of intense interest in cancer research, and it is not difficult to predict that differentiation markers identified by mouse monoclonal antibodies will have a profound influence on all work on human cancer—from providing new probes for analyzing the process of normal and abnormal differentiation to defining new markers for diagnosis and new ways to classify cancer, to identifying new targets for cancer localization and therapy. Perhaps the proudest justification of the commitment Lloyd Old and his friends have made to the development and characterization of mouse monoclonal antibodies has come from the recent demonstration that treatment with an antibody reactive with a cell surface ganglioside expressed in high concentration on melanoma cells induces regression of metastases in patients with advanced melanoma.

Application of the hybridoma technology to the production of human monoclonal antibodies lagged initially behind the developments in mice. Fortunately, the situation is changing rapidly and Lloyd Old's group has again taken the lead in developing and characterizing human monoclonal antibodies reactive with cell surface antigens and intracellular antigens of human cancer. Human monoclonal antibodies to human cancer antigens will undoubtedly have considerable value for a range of clinical applications. A critical advantage in their *in vivo* application is the lack of strong immunogenicity that hinders the usefulness of mouse monoclonal antibodies. What is most important, however, is that the humoral immune response to human cancer can now be examined in fine detail by hybridoma technology, allowing us to go beyond autologous typing in determining the range of cancer antigens that can be recognized by humans. This information is essential if active immunization with cancer vaccine is to be placed on a rational and firm basis, a consideration which brings us again to the subject of cancer therapy and to another topic which has held Lloyd Old's interest since his earliest days in research.

There are reports, reaching back even to the last century, of tumor regression in patients with concurrent infections. These observations led to attempts at treating cancer patients with bacterial toxins, a form of therapy which fell into disuse with the advent of radiation therapy and later chemotherapy. Interest was kept alive in the laboratory, however, and two lines of inquiry, not seen to be related at the time, led to renewed interest in microbial products as therapeutic agents. One had to do with the striking phenomenon of tumor hemorrhagic necrosis induced by gram-negative bacteria, later shown to be due to bacterial endotoxin. The other set of observations, ex-

emplified by Lloyd Old's work with BCG, was that mice pretreated with these materials showed heightened resistance to the growth of tumors. From the earliest work with BCG, it has been thought that macrophages are critical in the antitumor effects. Whether activated macrophages exert their effect by direct cell contact or by release of a soluble factor is still a subject of debate. But there is no doubt that cytotoxic factors are released by macrophage cultures after exposure to endotoxin. Lloyd Old and his colleagues have shown that a common factor may link endotoxin, BCG, tumor hemorrhagic necrosis and activated macrophages. The basic observation is that the serum of BCG-infected mice injected with endotoxin contains a factor that causes hemorrhagic necrosis of mouse tumors *in vivo* and tumor cell inhibition or destruction *in vitro*. The factor mediating this effect has been called tumor necrosis factor or TNF. TNF is also produced by human B-cell lines in culture, and has been shown to inhibit or destroy human cancer cells, but not normal cells, *in vitro*. In the fall of last year, the gene for human TNF was cloned in the laboratories of several biotechnology companies and expressed in *E. coli*, and we are of course excited about the prospect of beginning clinical studies of TNF in the near future.

After what I have said so far, it will surprise no one to hear that the impact of Lloyd Old's life as a scientist has not been limited to his own laboratory. He has received many honors in this country and abroad. Among them are a lecture to the Harvey Society, the Clowes memorial Lecture to the American Association for Cancer Research, the DeVilliers Award and election to the National Academy of Sciences. His advice and guidance in planning scientific strategy has been valued highly by our own institution, which he served as vice president and associate director for scientific development for 10 years, and by organizations concerned with supporting cancer research. Lloyd Old has served as Medical Director of the Cancer Research Institute, whose primary objective is to support the development of immunological approaches to cancer, and as scientific director of the Ludwig Institute for Cancer Research, whose purpose it is to originate and conduct incisive long-range research programs in cancer in conjunction with established medical centers in Europe, England, Canada and Australia, a responsibility which represents a truly global outreach. With all these activities, Lloyd Old has remained firmly anchored in his own laboratory, a generous though demanding teacher and a loyal friend to all of us who have had the good fortune of working with him.

I am honored and privileged to present him with the Academy medal tonight.